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FINAL TECHNICAL REPORT (NAGW-1382, and 1 year extension NAG10-0088)

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Our goal in carrying out this research was to investigate a model for plant growth regulation in which gravity-induced, asymmetric changes in the activities of two types of cell wall-localized enzymes might result in differential growth across an organ (stem, root, etc.) leading to a gravitropic bending response. Specifically, the two enzymes we investigated were 1) amine oxidases and 2) peroxidases. We envision a mechanism by which amine oxidase-generated H_2O_2 would be coupled to peroxidase-mediated cross-linking of cell wall constituents. This cross-linking would decrease cell wall extensibility and inhibit growth locally.

As a first step, we demonstrated that amine oxidase activities are indeed cell wall-localized. This was conclusively shown using an electron microscopic localization method that we developed in our lab (Slocum and Furey 1991). Gravitropic bending in corn coleoptiles is not accompanied by an asymmetric distribution in amine oxidase activities, although there is an asymmetric distribution of their amine substrates, which accumulate on the upper, slower-growing sides of the organ. We had previously demonstrated that Ca^{2+} also accumulated in the cell walls of tissue on the slower growing sides of gravistimulated coleoptiles (Slocum and Roux (1983) *Planta* 157: 481) and we have recently found that Ca^{2+} stimulates amine oxidase activity *in vivo*. This probably results from increased synthesis or secretion of the oxidase, since we have shown that Ca^{2+} do not stimulate amine oxidase activity *in vitro*. Regardless of the precise mechanism involved here, asymmetric amine/ Ca^{2+} gradients in gravistimulated tissues may set up H_2O_2 gradients and asymmetric peroxidase cross-linking profiles.

Most of our work in the past year, however, has focussed on the control of the peroxidases themselves. We have identified several cationic and anionic peroxidase (POD) isozymes which are differentially expressed in gravistimulated tissues and are, therefore, candidates for involvement in the asymmetric growth mechanism discussed above. Time course studies of their expression have demonstrated that the activities of some of these PODs are increased within 30 min. after the gravity stimulus is perceived. Like amine oxidase activity, the activities of several of these PODs are also modulated by Ca^{2+} and the plant hormone auxin. Auxin promotes growth of tissues and influences POD activity profiles in a manner that suggests Ca^{2+} and auxin may have antagonistic effects on growth, mediated largely at the level of PODs. We have partially-purified one POD corn coleoptile isozyme (designated A3) and are attempting to purify it to homogeneity so that we can raise antibodies against this protein. This will permit us to carry out high-resolution studies examining the spatial and temporal patterns of A3 expression in relation to gravitropic bending. These studies are in progress and several papers describing the most recent work are in preparation (see below).

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Publications

- Slocum, RD and Moyant, EC (1989) Characterization of polyamine oxidase activity in gravistimulated corn coleoptile. *ASGSB Bull.* 3: 98
- Slocum, RD and Nichols, HF (1990) The role of Ca^{2+} in the regulation of peroxidase activities and growth in corn coleoptiles. *ASGSB Bull.* 4: 20.
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- Slocum, RD and Furey, MJ, III (1991) Electron-microscopic cytochemical localization of diamine and polyamine oxidase in pea and maize tissues. *Planta* 183: 443-450.
- Slocum, RD (1991) Tissue and subcellular localization of polyamines and enzymes of polyamine metabolism. Pp. 93-103 *In*: Slocum, RD and Flores, HE, eds. *Biochemistry and Physiology of Polyamines in Plants*, CRC Press, Boca Raton, FL.
- Slocum, RD and Nichols, HF (1991) Asymmetric IAA oxidase activities accompany gravitropic bending in corn coleoptiles. *ASGSB Bull.* 5: 77.
- Slocum, RD and Nichols, HF Cell wall-localized amine oxidase activities and their possible role in the tropistic bending response. *Plant Physiol.* (submitted)
- Slocum, RD and Nichols, HF Calcium- and auxin-regulated isoperoxidase expression in corn coleoptiles. *Physiol. Plant.* (In preparation)
- Slocum, RD and Nichols, HF Rapid changes in isoperoxidase activities precede differential growth in gravistimulated corn coleoptiles. *Physiol. Plant.* (In preparation)